Flavonoids from the flowers of *Nymphaea alba* L.

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Abstract

Ten flavonoids were obtained from the flowers of *Nymphaea alba* L. Their structures were determined mainly on the basis of spectral analyses (UV, ^1H NMR, MS). The following aglycons were isolated: quercetin, kaempferol, isokaempferide and apigenin as well as the following glycosides: quercetin 4'- β -xyloside, 3-methylquercetin 3'- β -xyloside and a mixture of quercetin 3-galactoside and 3-glucoside. The structures of three compounds obtained in very small amounts were determined in part.

Key words: *Nymphaea alba* L., *Nymphaeaceae*, water-lily, European white water lily, flowers flavonoids

INTRODUCTION

Nymphaea alba L. is an aquatic perennial under partial protection in Poland. This species from the family *Nymphaeaceae* is characteristic for the *Nuphareto-Nymphaeetum albae* community. The chemical composition of the species under discussion has not yet been fully determined. The occurrence of alkaloids (Bułajewski 1936, Hegnauer 1969, Goleniewska-Furmanowa 1970, Glasby 1975), tannins (Hegnauer 1969), the glycoside nymphalin (Bułajewski 1936) and β -sitosterol (Joshi et al. 1974) has been determined in *N. alba*. There is only fragmentary information on the presence of flavonoids in this species (Hegnauer 1969, Taku et al. 1970) or such conclusions are based only on the results of chromatographic analyses (Wohlfart and Gademann 1974).

Interest in *N. alba* flavonoids arises from the fact that these compounds play a role in the chemical taxonomy of plants (Harborne 1986, Harborne et al. 1986, Merfort et al. 1986, Emerenciano et al. 1987) and also exhibit biological activity (Havsteen 1983).

MATERIAL AND METHODS

Plant material. The flowers were collected, with the approval of the local environmental protection authorities, in the old river bed of the Warta River in the vicinity of the town of Dębna, province of Nowe Miasto on Warta. The herbarium specimen was deposited at the Chair of Pharmaceutical Botany, Medical Academy, specimen number: 47569.

Chromatographic analyses. Various chromatographic techniques were employed in this study (Jerzmanowska 1967, Harborne 1973, Stahl and Schild 1981). Thin-layer chromatography (TLC) was used to check the distribution of the compounds contained in the particular fractions, when selecting the parameters for column chromatography (CC), in analysing degradation products and for co-chromatography with standards. Ready-made plates (Merck) with cellulose, 11 F254 polyamide and silica gel 60 were used. Ascending paper chromatography was carried out on Whatman No. 1 paper. Preparative isolation of compounds was done on plates covered with the appropriate adsorbent or Whatman No. 3 paper. Cellulose (Schleicher-Schüll) and polyamide (Mecherey-Nagel) were used in CC. The final stages of isolation of these compounds were carried out on Sephadex LH-20 (Pharmacia).

Chromatography systems. Cellulose or paper I — H_2O , II — 15% acetic acid (AcOH), III — 50% AcOH , IV — benzene (C_6H_6)- $\text{AcOH-H}_2\text{O}$ (125:72:3), V — C_6H_6 - $\text{AcOH-H}_2\text{O}$ (6:7:3, organic phase), VI — H_2O -saturated phenol; polyamide — VII — chloroform (CHCl_3)-methanol (MeOH)-methyl ethyl ketone (MeCOEt , 9:4:1), VIII — CHCl_3 - MeOH-MeCOEt (15:4:2); silica gel — IX — n-propanol-ethyl acetate- H_2O (7:2:1), X — CHCl_3 - $\text{MeOH-H}_2\text{O}$ (6:4:1). The distribution of the flavonoids and sugars on the chromatographs was compared with that of standards.

Isolation of flavonoids. Flavonoids were isolated using a commonly applied method (eg. Harborne 1973, Harborne and Mabry 1982). Two kg of fresh flowers (including the calyx but lacking stems) were separated into pieces and extracted together 4 times with boiling MeOH . Each extraction was carried out for 5 hours. The combined extracts were concentrated under reduced pressure until 111.6 g of a sticky residue remained. Further steps including the use of ethyl ether, Et_2O (9.6 g) and ethyl acetate AcOEt (23.0 g) led to the isolation of fractions containing flavonoids. Both of these fractions (5 g of Et_2O and 10 fractions containing flavonoids. Both of these fractions (5 g of Et_2O and 10 AcOEt) were used for the isolation of the reported compounds. Initial

Table 1
Characteristics of flavonoid glycosides isolated from flowers of *Nymphaea alba* L.

Compound	Mobile phases I-III	UV, λ MeOH max, nm		^1H NMR, δ , ppm	MS, m/e (% rel.)	Products of hydrolysis: aglycons (UV, TLC) sugars (TLC)
		s-shoulder	d-decreased			
1 3-Glucoside of 3,5,7,3',4'-pentahydroxyflavone (Isoquercitrin) and 3-Galactoside of 3,5,7,3',4'-pentahydroxyflavone (Hyperoside)	0.68 0.05 0.39	NaOMe NaOAc NaOAc/H ₃ BO ₃ AlCl ₃ AlCl ₃ /HCl	255s, 268, 288s, 351 274, 328, 405d 275, 325, 389 265, 299, 371 277, 302, 436 274, 297, 360, 403		303 (M + H, 18) 302 (M, 100) 153 (A ₁ , 7) 137 (B ₁ , 10)	quercetin glucose galactose
2 4'- β -Xyloside of 3,5,7,3',4'-pentahydroxyflavone (4'- β -xyloside of quercetin)	0.43 0.09 0.19	NaOMe NaOAc NaOAc/H ₃ BO ₃ AlCl ₃ AlCl ₃ /HCl	251, 266s, 322s, 370 282, 325, 427 275, 318, 400 254, 268s, 374 263, 308s, 354, 426 262, 271s, 307s, 354, 426	7.95/1H,d,J=2Hz/H-2' 7.82/1H,dd,J=9Hz,J=2Hz/H-6', 7.00/1H,d,J=9Hz/H-5' 6.44/1H,d,J=2Hz/H-8 6.20/1H,d,J=2Hz/H-6 4.75/1H,d,J=9Hz/H-1'' 3.40/m/H-2'',3'',4'',5'',6'',H ₂ O	303 (M + H, 16) 302 (M, 100) 301 (M-H, 17) 153 (A ₁ , 11) 137 (B ₁ , 13)	quercetin xylose
3 3'- β -Xyloside of 5,7,3',4'-tetrahydroxy-3-methoxyflavone (3'- β -xyloside of 3-methylquercetin)	0.59 0.13 0.61	NaOMe NaOAc NaOAc/H ₃ BO ₃ AlCl ₃ AlCl ₃ /HCl	249s, 268, 312s, 351 274, 329, 402s 275, 326, 400 269, 312s, 356 260s, 276, 301, 357, 403 258, 277, 298s, 352, 401	7.84/1H,d,J=2Hz/H-2' 7.67/1H,dd,J=9Hz,J=2Hz/H-6' 6.99/1H,d,J=9Hz/H-5' 6.44/1H,d,J=2Hz/H-8 6.19/1H,d,J=2Hz/H-6 4.79/1H,d,J=7Hz/H-1'' 3.80/3H,s/OCH ₃ 3.70-2.90/m/H-2'',3'',4'',5'',H ₂ O	316 (M, 1) 315 (M-H, 2) 153 (A ₁ , 8)	3-methyloquercetin xylose

separation was achieved on cellulose columns eluted with water, followed by a gradient of methanol-water (the methanol concentration increasing by 5%) from 5-50%, followed by 95% methanol. In addition to a mixture of phenylchromons, 2.5 g of nonflavonoid compounds (PA) were obtained from fraction Et20.

The flavonoids contained in the methanol eluates were then separated on polyamide using a methanol-acetone mixture containing increasing proportions of acetone: 5%, 50%, 75% and pure acetone. The eluates were analysed chromatographically (systems I and II). Unhomogeneous fractions were rechromatographed after which they were separated preparatively (systems III, IV, VII).

The compounds obtained in this manner were purified on Sephadex columns (1 × 10 cm). Crystalline or amorphous flavonoids in amounts from 0.1-27 mg were obtained by concentrating the eluates.

Identification of compounds. Uncorrected melting points (m.p.) were determined with a Boetius apparatus. Spectra were recorded and interpreted on the basis of data from literature (Mabry et al. 1970, Harborne and Mabry 1982, Gunther 1983). UV-Specord M-40, Zeiss; ^1H NMR in DMSO- d_6 , DMSO- $d_6 + \text{D}_2\text{O}$, δTMS , 89.6 MHz, using a Jeol FX 90 Q; EI-MS 75eV, 300 mA, using a Jeol M-D 100, the compounds in glass capillaries were placed directly in the ion source. The flavonoid glycosides were also subjected to partial and complete acid hydrolysis in aqueous 1M hydrochloric acid. The aglycons were extracted from the reaction mixture with ethyl ether. The extracts were washed with water until they were no longer acidic, dried over anhydrous sodium sulphate after which the ether was distilled off. The residue was subjected to analysis. Aqueous fractions were concentrated under reduced pressure at 40°C until a dry residue was obtained which was then dissolved in methanol and chromatographed along with sugar standards (systems VI, IX, X). The characteristics of the isolated glycosides are presented in Table 1.

RESULTS AND DISCUSSION

Two fractions containing flavonoids, Et₂O and AcOEt, were prepared from the flowers of the European white water lily. Preliminary qualitative analysis of these two fractions by means of two-directional TLC in mobile phases III and IV showed analogical sets of compounds. The fractions were separated on columns and preparatively using various types of adsorbents. As the final result, 10 compounds in crystalline and amorphous form were isolated. Their identification was based on spectral analysis (UV, ^1H NMR, MS). The products of complete and partial acidic degradation of glycoside bonds were also studied (compounds 1, 2, 3 Table 1). Taking into account the

amount of flavonoids obtained, it can be concluded that such aglycons as quercetin, kaempferol, 3-methylkaempferol (isokaempferide) and apigenin dominate in the flowers of white water lilies. Only the presence of monoglycosidic bonds was found (Table 1). Compound **1** was found to be a mixture of quercitin 3-galactoside and 3-glucoside (isoquercitrin and hyperoside). Their identification was made on the basis of spectral analysis (UV, MS) and on the basis of analysis of the products of partial and total acidic hydrolysis. The low R_f values in systems I and II also indicate monosidic structures of above-mentioned flavonols. The difficulties inherent in their separation are reported in several papers (eg. Harborne and Mabry 1982, Budzianowski et al. 1990).

Two more glycosides isolated from the flowers of *Nymphaea alba*, quercetin 4'- β -xyloside (compound **2**), 3-methylquercetin 3'- β -xyloside (compound **3**) deserve more attention. When the name of the aglycone is taken into account, the latter compound is called scoparol 3'- β -xyloside (Wollenweber and Dietz 1981).

The glycosides named above along with isokaempferide and apigenin, whose structures are presented in this paper, are compounds that have for the first time been isolated from the species, *Nymphaea alba*. (Fig. 1).

Flavonoid xylosides belong to compounds that are not common in plants; if they do occur it is usually in the form of kaempferol and quercetin derivatives with the sugar moiety at C-3 and C-7 (Harborne and Mabry 1982). Characteristic flavonoids for species from the genus *Juniperus* turned out to be xylosides of luteolin and scutelarein with the sugar moiety at C-6 (Lamer-Zarawska 1983). The scoparol 3'-xyloside that was obtained in this study had hitherto been identified only in the plant, *Dacrycarpus dacrydioides* A. Rich (*Podocarpaceae*) (Markham and Whitehouse 1984).

The quercetin 4'-xyloside is probably a new flavonoid (Harborne 1988). Three more flavonoids were obtained from *Nymphaea alba*, but in very small amounts (0.1-2 mg). Only UV spectra and acid hydrolysis was carried out on them. These compounds did not release sugars. Two of them are probably: 5,7,4'-trihydroxy-8-methoxy and 5,7,4'-trihydroxy-3,8,3'-trimethoxy flavones, which is supported by literature data (Voirin 1983, Wollenweber and Dietz 1981). The third compound may be supposed to be a quercetin derivative substituted at C-7.

In comparison with the plant, *Gutierrezia microcephala* (DC) A. Gray (Fang et al. 1986), the results of our studies show that the flowers of *Nymphaea alba* have a scanty flavonoid composition.

Flavonoids belong to a group of polyphenolic compounds that are used in the chemical taxonomy of plants (Hegnauer 1986). Because the family *Nymphaeaceae* is a taxon that has only been partially described chemically, the results obtained in this study may be found to be important traits of the studied taxon.

Isokaempferide *Apigenin* *Isoquercitrin* *Hyperaside* *Quercetin 4'-beta-xyloside* *3-methylquercetin 3'-beta-xyloside*Fig. 1. Structure of some flavonoids obtained from *Nymphaea alba* L. flowers

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Flawonoidy z kwiatów *Nymphaea alba* L.

Streszczenie

Ze świeżych kwiatów grzybieni białych otrzymano wyciąg metanolowy, z którego przygotowano dwie frakcje przy użyciu eteru etylowego i octanu etylu. Z frakcji wyodrębniono flawonoidy, które zidentyfikowano na podstawie wyników analiz spektralnych. Wydzielono aglikony flawonoidowe: kwercetynę, kemferol, izokemferol i apigeninę oraz 3'-ksylozyd skoparolu, 4'-ksylozyd kwercetyny oraz mieszaninę izokwercetyny i hyperozydu. Poza kwercetyną i kemferolem pozostałe związki wykryto po raz pierwszy w kwiatach grzybieni białych. Ksylozyd skoparolu wyodrębniono dotąd tylko z jednego gatunku roślin. Drugi ksylozyd jest prawdopodobnie nowym flawonoidem.